RESEARCH ARTICLE

Chlorambucil plus rituximab with or without maintenance rituximab as first-line treatment for elderly chronic lymphocytic leukemia patients

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In a phase II trial, we evaluated chlorambucil and rituximab (CLB-R) as first-line induction treatment with or without R as maintenance for elderly chronic lymphocytic leukemia (CLL) patients. Treatment consisted of eight 28-day cycles of CLB (8 mg/m^2/day, days 1–7) and R (day 1 of cycle 3, 375 mg/m^2; cycles 4–8, 500 mg/m^2). Responders were randomized to 12 8-week doses of R (375 mg/m^2) or observation. As per intention-to-treat analysis, 82.4% (95% CI, 74.25–90.46%) of 85 patients achieved an overall response (OR), 16.5% a complete response (CR), 2.4% a CR with incomplete bone marrow recovery. The OR was similar across Binet stages (A 86.4%, B 81.6%, and C 78.6%) and age categories (60–64 years, 92.3%; 65–69, 85.2%; 70–74, 75.0%; ≥75, 81.0%). CLB-R was well tolerated. After a median follow-up of 34.2 months, the median progression-free survival (PFS) was 34.7 months (95% CI, 33.1–39.5). *TP53* abnormalities, complex karyotype, and low *CD20* gene expression predicted lack of response; *SF3B1* mutation and *BIRC3* disruption low CR rates. *IGHV* mutations significantly predicted PFS. R maintenance tended towards a better PFS than observation and was safe and most beneficial for patients in partial response and for unmutated *IGHV* cases. CLB-R represents a promising option for elderly CLL patients.
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Introduction

Over 40% of patients with chronic lymphocytic leukemia (CLL) are diagnosed at ≥75 years and over 25% at 65–74 years [1]. Elderly patients have been consistently underrepresented in clinical trials, as age-related comorbidities may violate inclusion criteria [2].

Current standard of care for physically fit patients with untreated CLL is fludarabine, cyclophosphamide, and rituximab (FCR) [3], which induces the longest progression-free survival (PFS) and overall survival (OS) [4]. Although in the CLL8 trial no difference was noted in terms of response and PFS, patients >65 years showed significantly higher rates of grade 3–4 hematologic toxicity and infections compared to younger patients and no advantage in OS due to FCR [4]. Moreover, only 10–11% of patients >70 years entered each arm, underlying that elderly CLL are often ineligible for fludarabine-containing therapies [4,5].

Additional Supporting Information may be found in the online version of this article.

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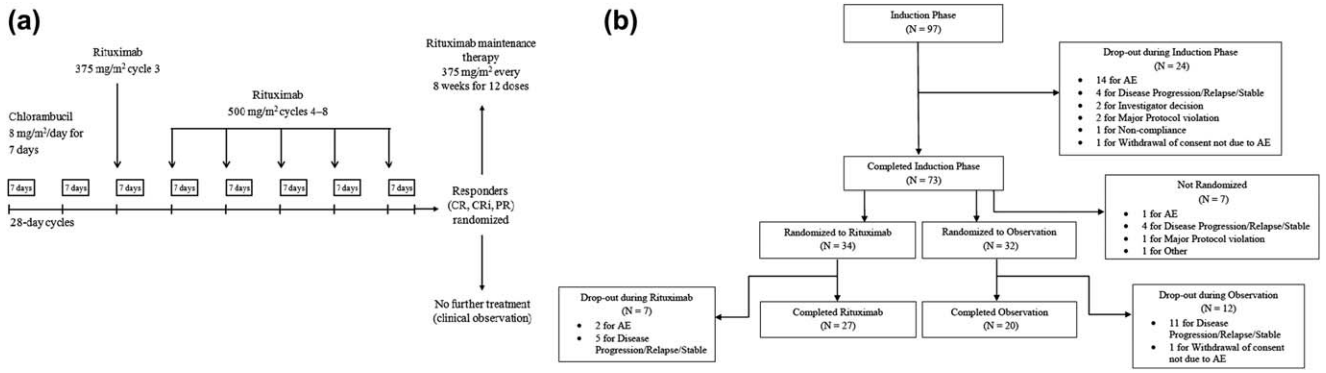

Figure 1. Treatment and patients flow. (a) Drug doses, schedules, and therapy scheme. CR = complete response; CRi = complete response with incomplete bone marrow recovery; PR = partial response. (b) CONSORT diagram. Summary of patients flow during the study.

Chlorambucil (CLB) remains therefore a widely used first-line treatment for such patients [3], but has limited effectiveness as monotherapy, with overall response (OR) rates up to 75%, but uncommon complete responses (CRs) and short PFS [6–9]. Management of elderly patients has remained a primary unmet need until recently, when evidence-based therapeutic strategies exploring the association between CLB and anti-CD20 monoclonal antibodies are being reported [10–12].

Several biologic properties of CLL cells allow patient stratification into risk categories that predict PFS and OS [4,13]. The impact of novel gene mutations (*NOTCH1*, *SF3B1*, and *BIRC3*) on CLL treatment outcome is currently under investigation.

We conducted a single-arm, phase II, open-label, multicenter study of CLB plus R (CLB-R) as induction, followed by a randomized maintenance with R or observation (protocol ML21445), to determine if CLB-R is feasible and beneficial as first-line treatment for elderly CLL patients. Treatment outcome has been correlated with biologic parameters, including gene expression profiling (GEP) and novel mutations. Furthermore, we explored the impact of R as maintenance treatment.

Methods

Patients. The study included patients aged >65 years (or 60–65 years not eligible for fludarabine-based regimens) with previously untreated CLL (Binet stage A or B with active disease or stage C), diagnosed according to the International Workshop on CLL (iwCLL) update of the National Cancer Institute (NCI) 1996 guidelines [14]. Exclusion criteria included: history of other malignancies within 2 years prior to study entry or severe cardiac disease, comorbidities requiring >1 month use of systemic corticosteroids, creatinine clearance <50 mL/min, or transformation to aggressive B-cell malignancy.

Study design and treatment. This study was designed to assess the efficacy and safety of CLB-R as induction therapy and explore the role of a maintenance phase with R or observation in responders. The primary endpoint was OR at the end of induction. Secondary endpoints included CR, CR with incomplete bone marrow recovery (CRi), and partial response (PR) at the end of the induction and OR, CR, and PR at the end of the maintenance phase, immunophenotypic CR, molecular CR, event-free survival (EFS), PFS, time to new CLL treatment (TTNT) or death, and OS, safety of the induction phase and the R maintenance arm, response and survival endpoints in biologically defined subgroups.

Response assessment was performed 2 months after induction completion. Definition of response was based on iwCLL criteria updating the NCI 1996 guidelines [14]. The first dose of R maintenance was scheduled 3 months after the last induction cycle in randomized patients.

The study was approved by the Institutional Review Board of the coordinating center and by the ethics committees of the 19 participating centers. All patients provided written informed consent. The trial was conducted in respect of the Helsinki Declaration, of Good Clinical Practice, and of applicable national regulations. The study period was from October 2008 to January 2013.

Treatment is detailed in Fig. 1a. Treatment modifications in case of cytopenia or adverse event (AE) are specified in Supporting Information.

Biologic work-up, including minimal residual disease (MRD) in CR patients, is detailed in the Supporting Information [15–22].

Statistical analysis. Based on an α-level of 0.05 to show a significant difference with respect to the primary endpoint of OR rate after the induction phase, the planned sample size has estimated from 72 to 94 subjects to obtain a power at least of 80% and a maximum of 90%.

The calculation of the sample size was based on the following assumptions:

- 0.65 OR proportion in null hypothesis.
- 0.80 OR proportion expected under alternative hypothesis.

Considering a 20% of non-evaluable subjects, from 90 to 118 subjects should have been included in the study.

Statistical testing was conducted at the two-sided $\alpha = 0.05$ level and two-sided 95% confidence intervals (CI) were used. To test the null hypothesis that the responders proportion was equal to 0.65 (H0: $\pi_t = 0.65$), the binomial distribution with the normal approximation was used (two-sided; $\alpha = 0.05$). A two-sided χ^2 test and two-sided 95% confidence limits for response were used. Multivariate logistic regression and univariate analyses assessed the impact of biologic parameters on response rates. The Kaplan–Meier approach was used for time-related variables estimates. Estimates for the median time-to-event and the corresponding two-sided 95% CI were made together with the estimates for the other quartiles and the range. Multivariate Cox regression analysis assessed the impact of prognostic factors on survival endpoints and the impact of treatment group and prognostic factors on survival endpoints on the randomized population (see Supporting Information).

Results

Patients' characteristics

Ninety-seven patients entered the induction phase (safety population) and 85 received ≥1 dose of R (intention-to-treat, ITT population). Upon induction completion, 34 patients were randomized to R maintenance and 32 to observation (Fig. 1b). Table I summarizes the characteristics of the ITT and the maintenance randomized population.

In the ITT population, the median age was 70 years (range: 61–84). Forty-four patients (51.8%) had ≥1 documented comorbidity at baseline: 6/13 patients aged 60–64 years (46.1%), 12/27 aged 65–69 (44.4%), 11/24 aged 70–74 (45.8%), 12/17 aged 75–79 (70.5%) and 3/4 aged ≥80 (75%).

Cytogenetic analysis using immunostimulatory CpG-oligonucleotide DSP30 plus interleukin-2 [16] identified 12 cases with complex karyotypes—that is, the presence of three or more cytogenetic aberrations in the same clone—5 of which devoid of 17p−/11q−.

Induction efficacy

The OR rate in the ITT population ($N = 85$) was 82.4% (95% CI, 74.25–90.46%, $n = 70$), with 16.5% ($n = 14$) CR, 2.4% ($n = 2$) CRi, 60.0% ($n = 51$) PR, and 3.5% ($n = 3$) nodular PR. The OR rate was similar across Binet stages—A, 86.4%; B, 81.6%; C, 78.6%—and age categories—60–64 years, 92.3%; 65–69 years, 85.2%; 70–74 years, 75.0%; ≥75 years, 81.0%. Two of four patients ≥80 years responded to induction. CR was achieved in 27.3% Binet stage A, 10.2% stage B, and 21.4% stage C patients. Upon induction completion, cytometric MRD on 14/16 CR/CRi patients showed a median of 0.02% residual

TABLE I. Patients Characteristics at Baseline (ITT Population) and at Randomization[a]

Characteristic	ITT		Rituximab		Observation	
	Patients, *n* or *n*/*N* (*N* = 85)	Patients, %	Patients, *n* or *n*/*N* (*N* = 34)	Patients, %	Patients, *n* or *n*/*N* (*N* = 32)	Patients, %
Age, years						
60–64	13	15.3	6	17.6	5	15.6
65–69	27	31.8	13	38.2	10	31.3
70–74	24	28.2	7	20.6	10	31.3
75–79	17	20.0	7	20.6	6	18.8
≥80	4	4.7	1	2.9	1	3.1
Sex						
Female	27	31.8	10	29.4	9	28.1
Male	58	68.2	24	70.6	23	71.9
B symptoms	24	28.2	8	23.5	9	28.1
Raised LDH	26/82	31.7	10/33	30.3	9/30	30.0
Lymphadenopathy	76	89.4	30	88.2	27	84.4
Splenomegaly	46	54.1	21	61.8	17	53.1
Hepatomegaly	14	16.5	4	11.8	8	25.0
ECOG performance status						
0	75	88.2	33	97.1	27	84.4
1	7	8.2	0	0.0	3	9.4
>1	3	3.5	1	2.9	2	6.3
Binet stage						
A	22	25.9	6	17.6	12	37.5
B	49	57.6	21	61.8	17	53.1
C	14	16.5	7	20.6	3	9.4
Comorbidities[b]						
Cardiovascular	32	37.6	15	44.1	11	34.4
Endocrine and metabolic	20	23.5	8	23.5	8	25.0
Genitourinary	8	9.4	4	11.8	3	9.4
Psychiatric disease	5	5.9	3	8.8	0	0.0
Serum parameters						
β-2M (>2 mg/L)	47/52	90.4	17/18	94.4	20/23	87.0
sCD23 (>70 UI/L)	75/78	96.2	31/31	100.0	29/31	93.5
TK (>7.1 U/L)	68/79	86.1	27/32	84.4	26/30	86.7
CD19/CD38+ (≥20%)	34/83	41.0	14/33	42.4	13	40.6
ZAP-70+ (≥20%)	63/83	75.9	26/33	78.8	21	65.6
IGHV unmutated	47/81	58.0	20/33	60.6	16/31	51.6
Hierarchical FISH-based chromosomal abnormalities						
TP53 disruption[c]	5/83	6.0	1/33	3.0	2	6.3
11q−	15/83	18.1	8/33	24.2	5	15.6
Trisomy 12	17/83	20.5	8/33	24.2	5	15.6
13q− only	25/83	30.1	9/33	27.3	11	34.4
No abnormalities	21/83	25.3	7/33	21.2	9	28.1
Novel gene mutations						
NOTCH1	12/82	14.6	3/33	9.1	6	18.7
SF3B1	11/82	13.4	4/33	12.1	4	12.5
BIRC3 disruption[d]	8/82	9.8	5/33	15.1	2	6.2

ECOG = Eastern Cooperative Oncology Group; FISH = fluorescence in situ hybridization; ITT = intent-to-treat; TK = thymidine kinase.
[a] Percentages are calculated on subject with sample performed and evaluable for each parameter.
[b] Patients with at least one comorbidity on the listed system. Comorbidities were assessed on the base of clinical charts and investigator's judgment.
[c] *TP53* disruption includes four patients with 17p− and one patient with *TP53* mutation without deletion.
[d] *BIRC3* disruption includes patients with *BIRC3* mutation and/or deletion.

CLL cells (range 0–4.8%) on peripheral blood (PB), with undetectable CLL in two cases. Bone marrow (BM) cytometric MRD showed a median of 0.34% residual CLL cells (range 0–4.3%), with undetectable CLL in two cases. None achieved a molecular CR.

Survival endpoints

The median follow-up of the ITT population was 34.2 months (range 3.0–43.5). The over 3-year PFS and EFS rates were 42.7% (95% CI, 27.3–57.4%) and 38.2% (95% CI, 24.4–51.9%), respectively (Fig. 2a,b). The median PFS and EFS were 34.7 (95% CI, 33.1–39.5) and 34.5 months (95% CI, 25.2–38.2), respectively. The median OS was not reached.

Induction safety

CLB was used at 56 mg/m^2 for 8 cycles (total dose: 448 mg/m^2). CLB dose reduction occurred in 51/657 cycles (7.8%), mostly for toxicity (40 cycles, 6.1%) and in 7/103 (6.7%), 18/210 (8.5%), 10/168 (5.9%), 14/136 (10.2%), and 2/40 cycles (5.0%) for patients aged 60–64, 65–69, 70–74, 75–79, and ≥80 years, respectively.

In the safety population ($N = 97$), 76 patients (78.4%) had at least one AE during induction: 84.6% in patients aged 60–64 years, 75% aged 65–69, 80.7% aged 70–74, 70.0% aged 75–79, and 90.0% aged ≥80. Thirty-three of ninety-seven patients (34%) experienced general disorders, the most frequent being pyrexia (12.4%), infusion-related reaction (6.2%), fatigue (5.2%), asthenia (4.1%), and chest pain and influenza-like symptoms (3.1%). Nineteen serious AEs (SAE) occurred in seventeen patients (17.5%) during induction: 30.7% aged 60–64 years, 14.2% aged 65–69, 15.3% aged 70–74, 5% aged 75–79, and 40% aged ≥80. The most common was anemia. Of them, five were CLB-related (herpes zoster infection, erythematous rash, lumbar pain, anemia, and fever of unknown origin) and three CLB-R-related (pleural effusion, anemia, and neutropenia). One fatal SAE during induction (renal failure and paralytic ileus) was not considered

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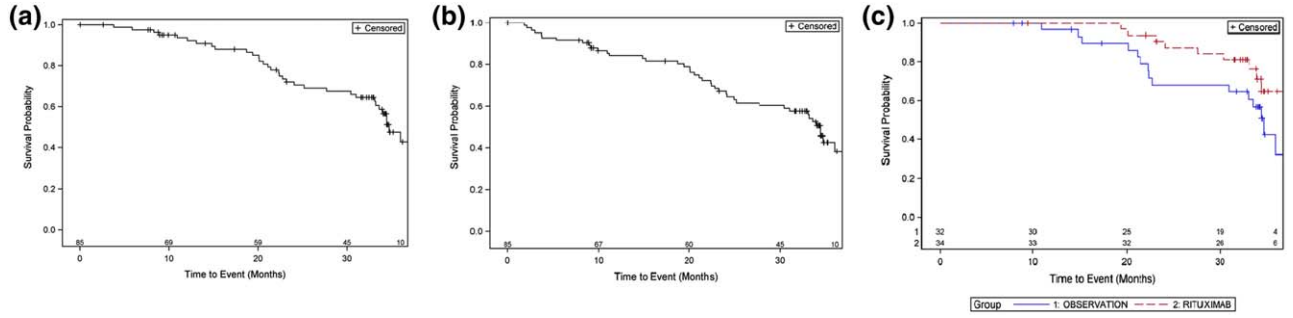

Figure 2. Survival endpoints. (a) Progression-free survival (PFS) of the ITT population. (b) Event-free survival (EFS) of the ITT population. (c) Progression-free survival (PFS) of the randomized population according to maintenance arm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

treatment related. Eight other deaths occurred after induction interruption: three from disease progression/relapse, one each from pulmonary infection, spinocellular carcinoma, anaplastic oligoastrocytoma, respectively, and two from unknown causes.

Maintenance

Among the 66 randomized patients (4 CR/CRi and 30 PR/nPR in the R arm, 12 CR/CRi and 20 PR in the observation arm), the OR rate after maintenance was 55.9% (95% CI, 39.19–72.57%) in the R arm and 34.4% (95% CI, 17.92–50.83%) in the observation arm, respectively ($P = 0.079$); the CR and PR rate were 29.4% and 26.4% in the R arm, and 18.7% and 12.5% in the observation arm, respectively. Notably, among the 50 PR/nPR patients randomized after induction, the proportion of responders to the R arm (17/30, 56.7%; 9 CR, 30%, 8 PR, 26.7%) was significantly higher than in the observation arm (5/20, 25%; 1 CR, 5%, 4 PR, 20%) ($P = 0.027$).

At R maintenance completion, 5/10 CR were evaluated for MRD: one proved MRD− in the PB and BM both immunophenotypically and molecularly, two were MRD− by flow in the PB and MRD+ in the BM; two were MRD+ in both compartments. All of them were in PR after induction (four PR, one nPR). At completion of the observation arm, none of the six CR patients was MRD−: four CR with MRD+ after induction experienced a MRD level increase, one MRD− CR after induction became MRD+, one PR after induction achieved a MRD+ CR.

In the randomized population, the median follow-up was 34.9 months (34.4 and 35.2 months for R and observation arm, respectively). The over 3-year PFS and median PFS rates were 48.6% and 38.2 months for R arm and 31.8% and 34.7 months for observation arm, respectively. There was a trend towards a longer PFS for patients receiving R maintenance ($P = 0.07$) (Fig. 2c).

In the R maintenance arm, 73.5% patients had at least one AE compared to 56.3% in the observation arm, with no significant difference ($P = 0.141$); no differential distribution of AE was recorded according to age. No difference in neutropenia ($P = 0.101$) or infections was recorded between the two arms (Table II). Ten SAE occurred in eight patients (12.1%) during maintenance, with an equal distribution according to arm and only one was R treatment-related (neutropenia). No fatal SAE occurred during maintenance; three patients (one in the R arm for lymphoma, two in the observation arm for disease progression/relapse and second tumor, respectively) died after maintenance interruption.

Biologic characteristics and patients' outcome

Univariate analysis in the population with response assessment ($N = 77$) evaluated the impact of prognostic factors on OR (Supporting Information Table SI) and CR (Supporting Information Table SII) achievement after induction and on PFS.

Only *TP53* disruption and a new model including complex karyotype within the high-risk FISH category (17p− and 11q−) were significantly associated to a poor response (each $P = 0.022$). The latter also predicted CR achievement ($P = 0.0225$). Contrariwise, 11q− was not associated to a poor response ($P = 0.403$): 13/15 patients (86.7%) achieved a response, including 3 CR and 10 PR.

Immunoglobulin heavy chain variable region gene (*IGHV*) mutations were the only significant predictor of PFS ($P = 0.0011$), with unmutated CLL having a 6.12 (2.070–18.077) higher risk of progression than mutated CLL.

Univariate analysis on the randomized population ($N = 66$) showed a significant difference in OR according to the *IGHV* mutation status in the observation arm (OR, 60% for mutated *IGHV* CLL vs. 12.5% for *IGHV* unmutated cases, $P = 0.009$) but not in the R arm (OR, 69.2% for mutated *IGHV* CLL vs. 45.0% for unmutated *IGHV* cases, $P = 0.284$), and according to the maintenance arm among unmutated *IGHV* patients ($P = 0.067$) but not among mutated *IGHV* CLL ($P = 0.705$). Consistently, a significantly different PFS within unmutated *IGHV* patients according to the maintenance arm was found ($P = 0.012$): the median PFS was 38.2 months (95% CI, 30.4–39.5) and 22.8 (95% CI, 20.1–33.1), respectively, for R and the observation arm.

A similar trend was observed for +12 CLL (OR, 66.67% in the R arm vs. 14.3% in the observation arm, $P = 0.06$). On multivariate analysis on the randomized population, unmutated *IGHV* independently predicted a significantly shorter PFS ($P = 0.0048$) and TTNT ($P = 0.0189$). Maintenance arm and 13q− showed a trend towards significance for PFS ($P = 0.0747$ and $P = 0.0512$, respectively).

Novel gene mutations and patients' outcome

Novel gene mutations were evaluable in 74 cases with response assessment. Nine patients were *NOTCH1* mutated: all responded to induction and 44% achieved a CR, with no significant difference with wild-type cases. Nine showed *SF3B1* mutations: 8/9 responded, not differently from wild-type cases; however, only 1/9 (11%) *SF3B1* mutated cases obtained a CR, as opposed to 14/65 (21.5%) wild-type cases. Similarly, 7/8 *BIRC3* mutated/deleted patients had an OR, with only 1 CR. For each mutation, PFS was assessed stratifying patients according to the presence of: (1) one of the novel mutations; (2) *TP53* disruption; (3) no mutations/disruptions. Only *SF3B1* mutations showed a trend towards a shorter PFS ($P = 0.0761$).

Neither in the R or observation arm, a significant impact on response was recorded according to the presence of mutations in 65 evaluable cases.

GEP and response to therapy

The GEP of 62 patients (CR/CRi = 16, PR = 41, no response [NR] = 5, including SD = 2 and PD = 3) was analyzed: samples did

TABLE II. Treatment Emergent AE During Induction and Maintenance

Adverse events	Induction				Rituximab				Observation			
	Any grade		Grade 3–4		Any grade		Grade 3–4		Any grade		Grade 3–4	
	Patients, *n*	Patients, %	Patients, *n*	Patients, %	Patients, *n*	Patients, %	Patients, *n*	Patients, %	Patients, *n*	Patients, %	Patients, *n*	Patients, %
Hematologic												
Blood and lymphatic system disorders	47	48.5	24	24.7	9	26.5	3	8.8	7	21.9	3	9.4
Anemia	15	15.5	3	3.1					1	3.1		
Hemorrhagic anemia	1	1.0	1	1.0					1	3.1	1	3.1
Leukopenia	5	5.2	3	3.1	1	2.9						
Neutropenia	32	33.0	19	19.6	5	14.7	3	8.8	1	3.1	1	3.1
Thrombocytopenia	16	16.5	1	1.0	1	2.9			1	3.1		
Non-hematologic												
Cardiac disorders	6	6.2							2	6.3		
Gastrointestinal disorders	17	17.5	2	2.1	3	8.8	1	2.9	4	12.5		
General disorders and infusion-related side effects	33	34.0	3	3.1	3	8.8			7	21.9		
Immune system disorders	2	2.1			1	2.9						
Infections	15	15.5	1	1.0	9	26.5			8	25.0		

Only AE judged clinically relevant and/or more frequent within safety population were selected.

not cluster according to their clinical responses either by unsupervised analysis or when comparing the three groups by ANOVA (not shown).

Contrariwise, the ANOVA analysis of CR versus NR patients (Supporting Information Fig. S1A) highlighted a homogeneous signature in the NR group, where two patients with concomitant 17p− and *TP53* mutation displayed the strongest signature. DAVID functional analysis revealed an overrepresentation of apoptotic ($P < 0.0001$) and anti-apoptotic pathways ($P < 0.0001$) (Supporting Information Fig. S1B).

Anti-apoptotic and pro-proliferative genes were more represented in the NR group, with the upmodulation of the Ras and Rho pathways; indeed, the upregulation of *KRAS*, *EP300*, and *NRAS* (Supporting Information Table SIII) was confirmed by quantitative PCR, particularly in the PD patients (not shown). NR patients also showed a significant downmodulation of the *CD20* transcript, that correlated with CD20 mean fluorescence intensity (Pearson correlation coefficient = 0.57). However, the correlation of the latter with response did not reach statistical significance (Supporting Information Fig. S2).

Among PR patients after induction who received R maintenance ($n = 21$), GEP analysis showed a clusterization according to their clinical response after maintenance, with a distinctive signature for the NR patients, again including the upmodulation of anti-apoptotic genes (Supporting Information Fig. S1C).

Discussion

The primary objective of this study was to evaluate the efficacy of CLB-R induction on OR achievement in elderly CLL patients. The trial provided very satisfactory results: the response rate—82.4% OR, including 16.5% CR and 2.4% CRi, with cytometric MRD− in 14% of CR cases—compares favorably with the 31–55% OR rates of trials using single-agent CLB at comparable doses [7,8,23], the 72% OR of the LRF CLL4 trial, using higher doses of single-agent CLB [6] and is supported by a similar British trial using CLB-R (OR 82%) [11]. Furthermore, in our study, PFS (34.7 months) is higher than that reported in trials using single-agent CLB (8.3–18 months) [6–8,12,23]. A preliminary analysis of the German CLL11 trial, comparing CLB alone, obinutuzumab + CLB, and CLB-R in untreated CLL patients with comorbidities, has been released [12]. The CLB-R arm provides inferior results (OR 65%, CR 7%, PFS 15.2 months) compared to ours, probably due to the lower CLB dosage/cycle and number of cycles. Interestingly, our OR and EFS results are also not inferior to those achieved by bendamustine plus R, although with less CR, and with a remarkably lower grade 3–4 hematologic toxicity [24]. Even though these comparisons have strong limitations due to different age inclusion criteria, CLB dosage, and treatment duration [9], it is worth noting that our study included patients with a median age of 70 years and is the only one including the maintenance with R [25–27].

At variance from fludarabine-based regimens, CLB-R was well tolerated: over three-quarter of patients completed all 8 induction cycles, regardless of age. CLB dose reduction was needed in 7.8% of cycles, due to toxicity in 6.1%.

In our study, AEs were generally hematologic, with neutropenia being the most common and affecting approximately one-third of patients in induction. Infections occurred in 15.5% of patients, although rarely of grade 3–4 (1%). Thus, CLB-R toxicity compares favorably with other trials using CLB alone or with R [6–8,11,23]. Infusion-related side effects were limited.

The role of maintenance in CLL is still an open issue, so far investigated by the use of monoclonal antibodies or lenalidomide. As in other chronic B-cell malignancies, the use of R after induction chemotherapy suggests a benefit in sustaining the response duration in CLL patients [28–31]. Most of the published studies used R after fludarabine-based regimens; the most recent one after FCR plus mitoxantrone, with a remarkable efficacy but a relevant hematologic

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and infectious toxicity [31]. Our study is the first to explore R maintenance after CLB-R induction. Although we could not formally demonstrate a clear advantage of R maintenance in responders after CLB-R induction, as maintenance was not the primary objective of the study, a better PFS was recorded compared to the control arm. In particular, PR/nPR patients after induction completion benefitted the most from R maintenance in terms of response improvement. Moreover, R maintenance determined a MRD clearing in induction responders in contrast to the observation arm, where MRD levels increased in all patients.

Remarkably, under R maintenance AE, SAE, neutropenia, or infections were as frequent as in the observation arm. Infections were the most common AE during maintenance, affecting one-fourth of patients, although never of grade 3–4. This supports the feasibility of maintenance strategies in elderly and/or unfit patients provided that the induction is not intensive and/or immunosuppressive [31].

Among several biologic markers, most had a limited value in predicting response to CLB-R, with the exception of genetic abnormalities. 17p−/*TP53* mutations and complex karyotype were significantly associated to a poor response and CR rate. Contrariwise, our data suggest that an induction therapy combining R and an alkylating agent may be beneficial for 11q− patients who are elderly and/or unfit for FCR [32].

Neither *NOTCH1*, *SF3B1*, or *BIRC3* mutations impacted on OR after induction, in line with other reports [33,34]; however, *SF3B1* mutations and *BIRC3* disruption were rarely detected in CR patients.

The only biologic marker significantly associated to a worse PFS was an unmutated *IGHV* status, although this did not impact on response to induction. Unmutated *IGHV* independently predicted a shorter PFS and TTNT also among randomized patients. However, the significant difference in response and PFS according to the *IGHV* mutational status found in the observation arm but not in the R arm suggests a benefit for unmutated *IGHV* in receiving R maintenance.

GEP may be useful in predicting response to induction and to R maintenance. NR patients in induction displayed a distinct signature from that of CR patients, with the concomitant downmodulation of pro-apoptotic and upmodulation of anti-apoptotic and pro-proliferative genes. Of interest is the overexpression of *KRAS* and *NRAS* [35], and the downmodulation of the *CD20* gene in NR patients. The latter observation, in line with in vitro [36] and in vivo data [31], suggests that higher doses or new anti-CD20 antibodies may be beneficial in biologically identified subsets of patients.

In conclusion, this study shows that: (i) CLB-R induction is an active first-line treatment for elderly CLL patients, regardless of age and disease stage; (ii) the toxicity is limited and manageable, regardless of age; (iii) PFS is promising and higher than that reported with CLB alone; (iv) CLB-R seems active also among 11q− patients; (v) NR patients may be identified by *TP53* abnormalities/complex karyotype, a distinctive GEP, and low *CD20* gene expression; (vi) *IGHV* mutations strongly influence PFS; (vii) low CR rates are recorded among *SF3B1* mutated and *BIRC3* disrupted cases. Moreover, we suggest that R maintenance is doable in elderly CLL patients and tends to improve PFS in patients responders to a CLB-R induction, not increasing toxicity compared to observation in terms of AE, SAE, neutropenia, or infections. It seems beneficial mostly in PR/nPR patients after induction in terms of response improvement and in unmutated *IGHV* patients. Therefore, we support the need of prospective phase III clinical trials to conclusively define the role of maintenance in maintaining response and prolonging survival also in elderly CLL patients. Rituximab, ofatumumab, and obinutuzumab are the candidates that deserve a comparison in this context.

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